



Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [9]:45-61
[<http://scholarsresearchlibrary.com/archive.html>]



Evaluation of Anti- Microbial Potential of Baeolepis Nervosa (Wight & Arn.)

Decne. Ex Moq.

Logesh R^{1*}, Dhanabal SP¹, Duraiswamy B¹, Rajeshkumar R¹, Chaitanya
MVNL¹, Rajan S²

¹Department of Pharmacognosy and Phytopharmacy (Off campus, Jagadguru Sri Shivarathreeswara University),
JSS College of Pharmacy, Rockland's, Ooty, India

²Center of Medicinal Plants Research in Homoeopathy, Ministry of AYUSH, Govt. of India, Emerald.

*Corresponding author: Logesh R, Department of Pharmacognosy and Phytopharmacy (Off campus, Jagadguru
Sri Shivarathreeswara University), JSS College of Pharmacy, Rockland's, Ooty, India. Tel:+91-9488134810; E-
Mail: rlogesh14@gmail.com

ABSTRACT

Objective: Baeolepis nervosa is endemic to Western Ghats, Nilgiris and is used by the tribes of nilgiri hill's namely "Irulas". The present study was done to evaluate the B. nervosa, a novel study of this plant.

Material and methods: The B. nervosa were undergone photomicrography, to study the microscopical character of the plant. To evaluate the B. nervosa for anti-microbial properties for both Bacteria and Fungi. The Fractionation was carried out using (Pet ether, Chloroform, ethyl acetate) the MeOH extract, and these fractions were carried out for anti-oxidant potentials.

Results: The transverse section of leaves and stem of B. nervosa has been analyzed and reported. The preliminary phytochemical analysis confirms the presence of alkaloids, flavonoids, phenols, steroids, tannis and triterpenes. The zone of inhibition for anti-microbial activity showed results only in gram -ve (E. coli) of B. nervosa leaf and in fungi it showed in A. niger of both B. nervosa leaf and stem. The aqueous extract showed good IC50 value for B. nervosa leaf (IC50 – DPPH- 52.75µg/ml, ABTS- 45.14 µg/ml, NO2- 64.32 µg/ml & H2O2- 70.80 µg/ml) and Ethyl acetate extracts showed good IC50 value for B. nervosa stem (IC50 – DPPH- 69.19 µg/ml, ABTS- 59.91 µg/ml, NO2- 54.30 µg/ml & H2O2- 56.06 µg/ml).

Conclusion: It can be concluded that B. nervosa has shown a good activity against anti-microbial as well as in anti-oxidant studies.

Keywords: Pharmacognostical, Extraction, Photomicrography, Antioxidant, Anti-bacterial, Anti-fungal.

INTRODUCTION

The Plant B. nervosa is narrowly endemic to Western Ghats, the Nilgiris and restricted to temperate regions of Blue Mountains of Tamil Nadu. It has been recorded at an elevation of 1500 to 2300 mts on rocky slopes and exposed barren land.

Plant Description

Baeolepis nervosa (Wight & Arn.) Decne. ex Moq. *Brachylepis nervosa* Wight & Arn. *Decalepis nervosa* ((Wight & Arn.) Venter. Sharma *et al.* in Biol. Mem. 2(1 & 2): 90. 1977 [Periplocaceae] (**Figure 1**).



Figure 1: Baeolepis nervosa plant aerial part.

Description A large climbing shrubs, stem purplish, pubescent; latex milky. Leaves simple opposite, 5-10 x 3-7 cm, thick, elliptic or elliptic-oblong, acute to acuminate, base obtuse, upper surface glabrous and shining; lateral nerves 8-10 pairs, prominent below, purplish in young leaves; petiole to 1 cm long. Flowers in axillary and terminal condensed cymes; peduncle pubescent to glabrescent. Calyx deeply lobed, lobes ovate, acute. Corolla rotate, lobes ovate greenish-purple; corona of 5 broad membranous scales. Stamens 5, attached to the throat of corolla; filaments short. Ovary glabrous; style apex capitate. Follicles narrowly lanceolate, 6-7 cm long with a long white coma.

Distribution Indigenous to India and narrowly endemic to Western Ghats; found in subtropical to temperate regions of the district from 1500 - 2300m. Altitude ranges.

Part Used Whole plant.

Folklore Uses The stem is tied around the wrist of the patient to cure rheumatism and arthritis (Johnson, 1991). The fresh latex is applied over boils and wart for ripening and quick healing. Used by Irulas. [9]

The Indigenous people or ethnic communities throughout the world have developed their own cultures, customs, religious rites, taboos, myths, folk beliefs, songs, food and plants to treat various diseases etc. The plant, which available in their environment will be used for their socio-cultural and medical practices, whether wild or cultivated. Knowledge is evolved over generation-to-generation of their experience and practices. Even though the modern civilization is penetrating into most of the tribal communities of the world, still the provincial health care practices are in vogue among some of the tribal communities in particular and also other population groups in general.

India is among one of the mega biodiversity in the world and having one or more type of forest ecosystem within. India is not only a biological diversity and also having cultural diversity encompasses a number of tribal communities in their different environmental conditions. The various ethnic communities are practicing health care system by using a variety of herbals for curative as well as preventive medicines. An all India coordinated research project on ethno biology was conducted under Man and the Biosphere programmed to identify the plant and animal wealth in tribal areas. These studies have shown that the tribal people for their various requirements use over 9500 plant species. For food (3900 species), medicinal purpose (7500 species), fiber and cordage (525 sp.), fodder (400 Sp.), gum, resin and dyes (300 sp.), material and cultural requirements (700 sp.) and so on. [2,3]

Traditional healing practices or native remedies are taken as an accurate reflection of crude drug resources of that country. The use of natural products as medicine could be traced back as far as the beginning of human civilization. The earliest mention of medicinal use of plants and animals is noticed in Rig-Veda, which is examined the oldest repository of human knowledge (4500–1600 BC). The various traditions, beliefs,

culture, religious practices of ethos and the diversity of flora and fauna in India richly contributed to folk medicine. [8] The Athervanaveda also describes the uses of medicinal plants. Indian Ayurveda medicine as described in the above treatise dates back to second millennium BC. The milestone developments in Indian medicine took place when Charaka Samhita (1000–800 BC) and Sushruta Samhita (800–700 BC) were prepared by the doyen of Indian medicine.

Nilgiri district is one of the richest diversity regions in India and particularly in the Western Ghats in temperate province. It is inhabited by the primitive ethnic groups as well as the traditional health care practices of this region. Sustainable use of folk medicinal plants (both ecosystem and traditional knowledge) requires a set of community-based strategies for the future generation.

MATERIALS AND METHODS

Collection of Plant Specimens

The plant *B. nervosa* was collected near Sholur reserve forest area, Udthagamandalam Taluk of Nilgiri district about 2000 mts altitude. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin- 5ml + acetic acid- 5ml + 70% Ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated in a graded series of tertiary-Butyl alcohol as per the schedule given by Sass (1940). Infiltration of the specimens was carried by the gradual addition of paraffin wax (melting point 58° - 60° C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

Paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. De-waxing of the sections was by customary procedure. The sections were stained with Toluidine blue as per the method. [7] Since Toluidine blue is a polychromatic stain. The staining results were remarkably good and some phytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary sections were also stained with safranin and Fast green and IKI (for starch). For studying the stomatal morphology, venation pattern and trichome distribution paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerin mounted temporary preparations were taken to ensure macerated/cleared materials. Powdered materials of different parts were eliminated with NaOH and mounted in glycerin medium after staining. Different cell component was studied and measured.

Photomicrograph

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations of bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bi-refrangent property under polarized light they appear bright against a dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books. [4, 5,6, 10,].

Extraction

Bacolepis nervosa leaf and stem were collected from the Sholur reserve forest area, Udthagamandalam, Nilgiri region in 2014-2015, throughout the month of August and authenticated in J.S.S college of pharmacy, Rockland, Ooty and vide voucher specimen number "8890" for future references. The leaf and stem parts were dried under shade and pulverized by using a mechanical grinder to make a coarse powder. Then coarse powder for leaf 980gm and stem 950gm were extracted with 95% methanol at room temperature (25°C). The leaf and stem extract were collected, filtered and

the solvent was evaporated to dryness under reduced pressure by using Buchi R-210 at 40-45°C. The concentrated methanol extract *B. nervosa* leaf (4.82% w/w) and *B. nervosa* stem (2.79% w/w) was transferred in an amber colored bottle and kept in a desiccator for further use.

Phytochemical Investigation

The methanolic extract of *B. nervosa* was subjected to phytochemical investigation to identify the various plant constituents like; Alkaloids, Carbohydrates, Fats and Fixed oils, Flavonoids, Glycosides, Lignin's, Phenols, Proteins and amino acids, Saponins, Steroids, Tannins and Triterpenes. [1, 10, 12].

Fractionation Method

The 20 gm of methanolic extract of *B. nervosa* (leaf and stem) was subjected to fractionation using Pet ether, Chloroform, Ethyl acetate and aqueous. The amount of solvent used was about 100 ml each and the fractionation was done in triplicate to get the extracts.

Anti-Microbial Assay

Preparation of stock solution of antibiotic, plant extracts and their biomarkers

Stock solution for *E. coli* – Tetracycline and *S. aureus* – Amoxicillin and for *C. alibicans* and *A. niger* – Amphotericin-B (Sisco Research Laboratory, India) were used as a concentration of 10 µg/ml (w/v). DMSO 1% (v/v) was used as solubilizing solvent for test samples and also used as control to evaluate the antimicrobial assay. Stock solution of *B. nervosa* leaf and stem were prepared and the final concentration of each plant extract was 5000 µg ml⁻¹, freshly prepared stock solution and requisite different concentration for the bacterial and fungal tests were prepared from this stock solution.

Microbial strains and culture condition

Gram positive (*Staphylococcus aureus* - ATCC 29213) and gram negative (*Escherichia coli* ATCC 25922) bacteria and Pathogenic (*Aspergillus Niger* - ATCC 16404) and Non- Pathogenic organism used was (*Candida alibicans* - ATCC 10231) were selected as standard strains as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI), formerly called National Committee for Clinical Laboratory Standards (CLSI, 2006) for experimental purpose bacterial cultures were maintained on Nutrient Agar (NA) or Nutrient Broth (NB) (Himedia, Mumbai, India) at 4°C and subculture in every 4 weeks.

Disc diffusion method

The antimicrobial assay of crude extracts and their biomarkers were performed by disc diffusion method (CLSI, 2006) Concisely, 10 ml of sterilized Muller Hinton Agar (MHA) (pH 7.2 ±0.2, at 25°C) were applied in to the surface of sterile Petri dishes (9 cm in diameter, Borosil) and allowing them to settle for base plate preparation. 100 ml of test bacterial and fungal suspension (5×10⁵ CFU/ml) were poured to each base plate and cotton swab (Himedia). 20 ml of different concentrations of each test sample (50-2000 mg/disc) were soaked with sterile paper discs (6 mm). The air-dried discs were placed on each base plate and incubated at 37 ± 2°C for 24 h. For bacteria - Tetracycline and Amoxicillin and fungi - Amphotericin-B was used in 20-mg/disc concentrations range as microorganisms respectively. The inhibition of zones around the discs was determined as the diameter (mm) of bacterial growth inhibition. The zone of inhibition was taken as an average of three measurements at different directions. [14] All experiments were performed in triplicate.

Determination of minimum inhibitory concentration (mics) and minimum bactericidal concentration (mbcs) and minimum fungicidal concentration (mfcs)

MICs values were determined by broth micro-dilution method suggested by (CLSI, 2006) Briefly, microbial cultures were prepared by suspending one isolated colony from each base plate in 5ml of MHB. After 24 h of proper incubation period, the suspensions were diluted in to get the final inoculum population (5×10^5 CFU/ml) by using to 0.5 Mac Farland standard. By colony morphological study were carried out for checking the accuracy of mother culture throughout the test. 96-well microtiter plates were used for two fold serial dilutions of test samples using known stock solution with MHB. An equal volume of bacterial and fungal inoculums were added to each well on the microtiter plate consist of 0.05 ml of serial dilutions of extracts which was incubated at $37 \pm 2^\circ\text{C}$ for 24 h. MICs values were defined as the lowest concentration of substance that inhibits visible growth of bacteria and fungus in media. Bacterial and fungal growth was displayed by the presence of turbidity and a pellet on the well bottom. MICs were determined presumptively as the first well, where no pellet appeared. [15] It was calculated by comparing the absorbance of sample wells with the control wells with the help of Spectra-max M5 (USA) at 405 nm wavelengths. The MBC and MFC was determined by adding 50 μl of the suspensions from the wells in 25 ml fresh MHB. These suspensions were re incubated at 37°C for 48 h. The MBC and MFC were determined as the lowest concentration of extract, which inhibited the complete growth (100%) of microorganisms.

Time course assay

The rapidity and duration of antibacterial and antifungal activity was determined by time-kill analysis. [16] Overnight broth cultures of bacterial and fungal strains were adjusted to the concentration of 5×10^5 CFU/ml and were treated with plant extracts (MIC $\times 2$). Control tubes were also prepared without plant extract. Then 100 μl of sample was taken and plated on MHA plates at regular time intervals (0, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, 60 h and 72 h). The plates were incubated at 37°C for 24 h and CFU were calculated. All the determinations were done in triplicates.

In Vitro Anti-Oxidant Studies***DPPH free radical scavenging activity***

The antioxidant activity of the sample was assessed on the basis of the radical scavenging effect of the stable DPPH free radicals, the DPPH free radicals are reduced to a corresponding hydrazine when it react with the hydrogen donors. The DPPH is purple and upon reaction with hydrogen donor's it becomes colorless. It is a discoloration assay, which is evaluated by the addition to a DPPH solution in methanol and decrease in absorbance was measured.

ABTS radical scavenging activity

This method involves the scavenging of ABTS [2,2' azino bis (3- ethylbenzthiazoline- 6- sulfonic acid) diammonium salt] radical cation. The principle behind the technique involves the reaction between ABTS and potassium persulphate to produce the ABTS radical cation, a blue green chromogen. In the presence of antioxidant reductant, the colored radical is converted back to colorless ABTS, the absorbance of which is measured at 734 nm.

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interact with oxygen to procedure with oxygen to produce nitric ions, which can measure by Griess reagent. Sodium nitroprusside spontaneously generate nitric oxide in aqueous solution. Nitric oxide generate in the manner was convert into nitric oxide and nitrous acids in contact with dissolved oxygen and water. The

liberated nitrous acid is estimated using Griess reagent, which forms a purple azodye. In the presence of test compound likely to the scavenger the amount of nitrous acid will decrease. The degree of decrease in the formation of the purple azodye will reflect the extent of scavenging.

H2O2 scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration²⁶. Aliquot of 1.0 mL of 0.1 mm H₂O₂ and 1.0 mL of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 mL of 2 M H₂SO₄ and 7.0 mL of 1.8 M KI. The mixed solution was titrated with 5.09mm Na₂S₂O₃ until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \times 100$$

Where, V₀ was volume of Na₂S₂O₃ solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V₁ was the volume of Na₂S₂O₃ solution used in the presence of the extracts.

RESULTS

Anatomy of Leaf

In sectional view, the leaf consists of a thick midrib with single are shaped vascular strand and thick lamina (Figure-1.1). Midrib has a thick and short adaxial cone and semi circular and wide abaxial part. The midrib is 1mm thick. The adaxial cone is 50 μm in height and 150 μm in width. The abaxial part 1 mm wide. The midrib has a thin and continuous epidermal layer of small, squarish thick walled cells. The ground tissue of the adaxial cow includes collenchymatous cells. The abaxial part consists of circular thin walled less compact parenchyma cells.

The vascular strand is wide and is shaped with adaxial concavity. The vascular strands are bicollateral, i.e. phloem occurs both on the adaxial concave side and abaxial convex side. The xylem elements include short, vertical parallel lines of narrow circular/elliptical vessels and sclerenchyma elements distributed in between in the vessel lines. Small circular units of phloem occur in discrete line. The phloem elements include narrow thick walled angular sieve elements and parenchyma cells (Figure 2.1). The vascular strew is 250 μm thick and 700 μm wide. The meta-xylem elements of the xylem strand are 20 μm in diameter.

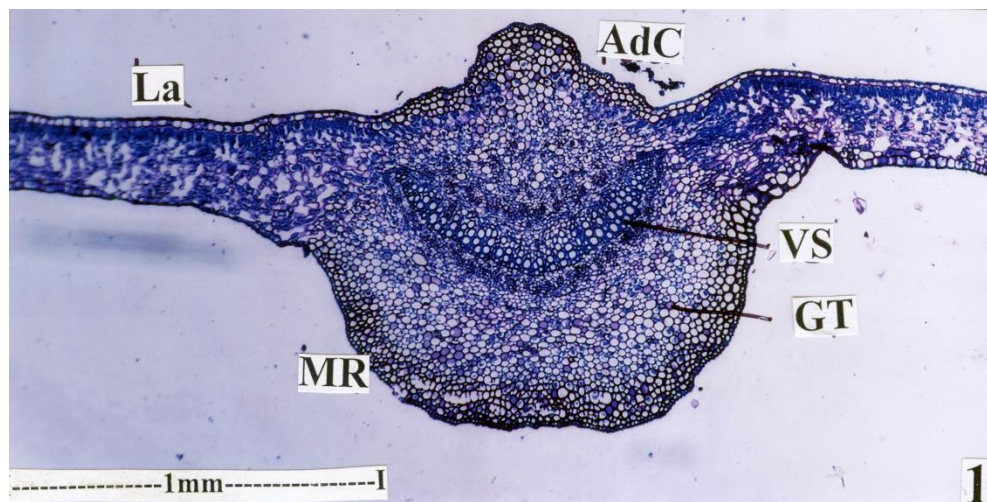


Figure. 1.1: Showing the T.S. of Leaf through Midrib.

Adc - Adaxial cone, GT - Ground Tissue, La - Lamina, MR - Midrib, VS - Vascular strand, Abph - Abaxial phloem, Adph - Adaxial phloem, Ep - Epidermis, Col - Collenchyma, X - Xylem.

Anatomy of Stem

The stem is circular in cross-sectional view with smooth surface. It is 2.3 mm in diameter. It consists of thick cortical zone, hollow thick vascular cylinder and wide central pith (Figure 2.1). The epidermis of the stem consists of small highly thick walled semicircular cells. The sub-epidermal layer of cells is filled with dense tannin content (Figure 2.2, 3). The cortical zone is wide and thick it is 100 μM thick in radial plane. The cortical tissue consists of homogeneous parenchyma cells, which are angular, compact and thick, walled. Along the inner boundary of the cortex occurs a cylinder of several discrete segments of gelatinous fibers (g-fibers). The fibers have lignified outer primary walls and mucilaginous (gelatinous) inner secondary walls (2.2, 3).

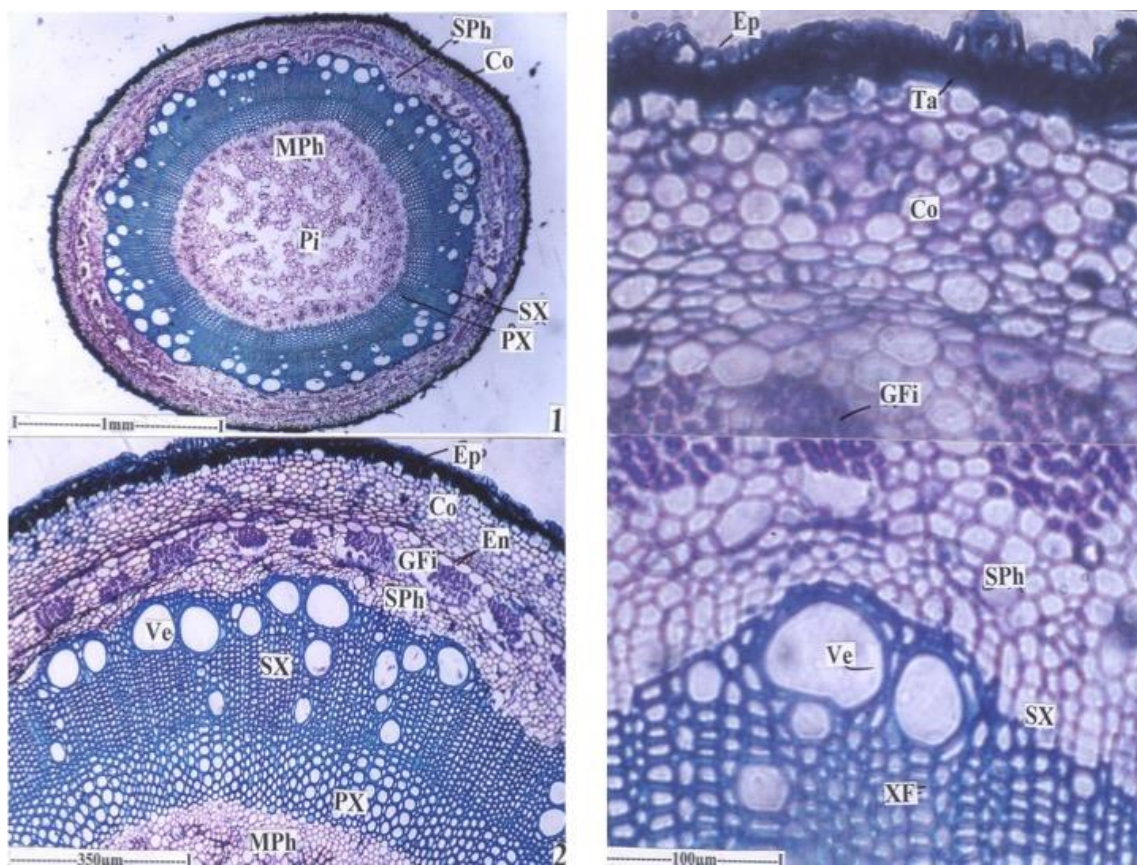


Figure 2.1: Showing the T.S. of stem entire view

Figure 2.2: Showing the T.S. of – T.S. of stems a sector

Figure 3: Showing the T.S. of stem a sector enlarged showing epidermis, cortex and Secondary xylem and phloem.

Co - Cortex, MPh - Medullary phloem, Pi - Pith, Px - Primary xylem, SPh - Secondary phloem, Sx - Secondary xylem, En - Endodermis, EP - Epidermis, GFi - Gelatinous fibre, Ve - Vessels. Ta - Tannin, XFi - Xylem fibres.

The vascular cylinder hollow and the cylinder consist of dense and compact phloem and xylem tissues. The cylinder is 400 μ M thick (Fig.-2.2). The vascular cylinder consists of outer continuous narrow cylinder of secondary phloem (outer phloem) and inner small nests of medullary phloem, which occur in a circle along the inner periphery of the xylem cylinder (Fig.- 2.2). The outer secondary phloem consists of thin layer crushed cells, which appears dark tangential lines. The inner part consists of intact sieve elements (Fig.-3.). Inner medullary phloem includes small units of sieve elements, which are quite adjacent to the xylem cylinder. The pith is wide and contains disintegrated and scattered parenchyma cells. Dispersed in the pith cells are scanty calcium oxalate druses.

Preliminary Phytochemical Screening

Preliminary Screening of various chemical compounds or Secondary metabolites in *B. nervosa* (leaf and stem) was under taken

S.No	PHYTOCHEMICAL TEST <i>B. nervosa</i>	RESULT	
		Leaf	Stem
1.	Alkaloids	+ ve	+ve
2.	Carbohydrates	+ ve	+ ve
3.	Fats and Fixed oils	- ve	- ve
4.	Flavonoids	+ ve	+ ve
5.	Glycosides	- ve	- ve
6.	Lignin's	+ ve	+ ve
7.	Phenols	+ ve	+ ve
8.	Proteins and amino acids	- ve	- ve
9.	Saponins	+ ve	+ ve
10.	Steroids	+ ve	+ ve
11.	Tannins	+ ve	+ ve
12.	Triterpenes	+ ve	+ ve

Antimicrobial Activity

MIC, MBC and MFC of plant extracts

B. nervosa (leaf & stem) extract were tested against bacterial and fungal (*E. coli*, *S. aureus*, *A. Niger* and *C. albicans*) strains showed significant inhibitory activity with MIC below 2000 $\mu\text{g ml}^{-1}$ (Table 1). The plant *B. nervosa* leaf and stem did not show inhibitory activity against *S. aureus* and while it showed inhibitory activity against *E. coli* with MIC 854 $\mu\text{g/ml}$ where as *B. nervosa* stem did not show any inhibitory activity against *E. coli* (Table 1). In fungal strains, *B. nervosa* leaf and stem showed inhibitory activity against *A. Niger* with MIC 714 and 805 $\mu\text{g/ml}$, respectively. Whereas *B. nervosa* stem did not show inhibitory activity against *C. albicans* (Table1). The results further demonstrated that the inhibitory activity of *B. nervosa* leaf against *E. coli* with MIC 854 $\mu\text{g ml}^{-1}$, respectively. Whereas *B. nervosa* leaf and stem did not inhibit the gram-negative bacterial strain (*S. aureus*) (Table 1) respectively. Hence, the results indicated that *B. nervosa* leaf showed (20 \pm 0.5 mm) antibacterial activity against *E. coli* and whereas in antifungal both *B. nervosa* leaf and stem showed activity against *A. Niger* (17.7 \pm 0.5 & 19.5 \pm 0.1 mm) respectively. Whereas, the *B. nervosa* leaf and stem extract possesses good (20 mm) antibacterial activity against *E. coli* and moderate to good (15-20 mm) antifungal strains *A. Niger*. The % inhibition of the *B. nervosa* leaf and stem against the tested bacterial and fungal strains are shown in Figure 1 (A & B). The minimal bactericidal concentration assay, using 2- to 3-fold MIC and minimum fungicidal concentration assay using 2-fold, but at higher concentrations had bactericidal and fungicidal activity (Table 2), due to the presence of one or more active principle in the extract.

S.No	Name of the Organism	Name of the plant extract			
		<i>B. nervosa</i> leaf		<i>B. nervosa</i> stem	
		MIC (μgml^{-1})	ZOI (mm)	MIC (μgml^{-1})	ZOI (mm)
1.	<i>E. coli</i>	854	20 \pm 0.5	-	-
2.	<i>S. aureus</i>	-	-	-	-
3.	<i>A. Niger</i>	714	17.7 \pm 0.5	805	19.5 \pm 0.1
4.	<i>C. albicans</i>	-	-	-	-

Table 1: Zone of Inhibition and MIC value of the plant extracts.

S.No	Plant extracts	MBC (μgml^{-1})		MFC (μgml^{-1})	
		<i>E. coli</i>	<i>S. aureus</i>	<i>A. Niger</i>	<i>C. albicans</i>
1.	<i>B. nervosa</i> leaf	1708	-	1428	-
2.	<i>B. nervosa</i> stem	-	-	1610	-

Table 2: MBC and MFC value of plant extracts.

Percentage Inhibition

% of inhibition for *B. Nervosa* Leaf and stem against *Aspergillus niger*

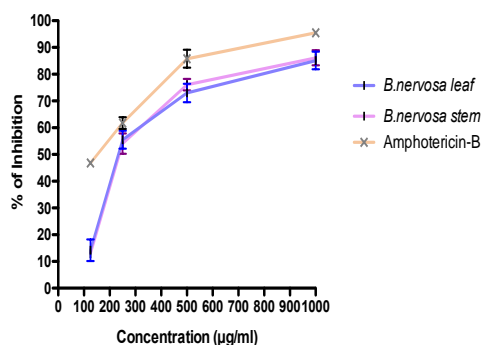


Figure 1A

% of inhibition for *B. Nervosa* Leaf against *E-coli*

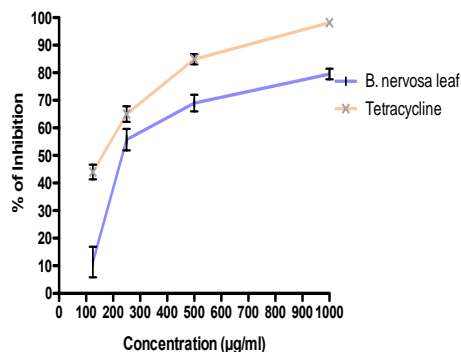
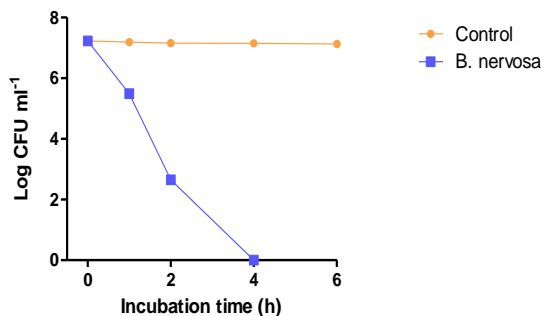
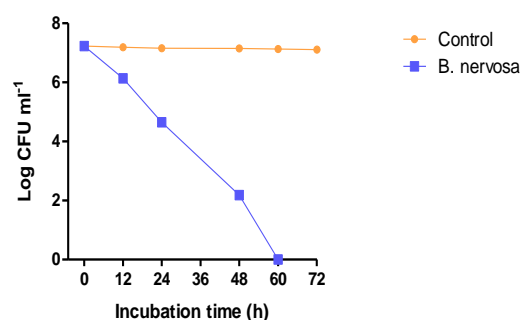


Figure 1B

Time course assay



Time course assay of *B. nervosa* against *E. coli*



Time course assay of *B. nervosa* against *Aspergillus niger*

Anti-Oxidant Studies

The antioxidant studies were carried out using *B. nervosa* (leaf and stem) fractions. The following methods were used to identify the antioxidant effects are DPPH, ABTS, Nitrous oxide and H₂O₂ and finally the percentage of inhibition and IC₅₀ values are calculated.

DPPH Method

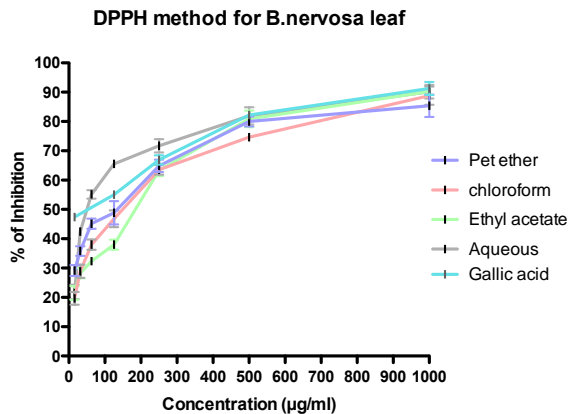


Figure: B. nervosa leaf

METHOD	FRACTIONS	IC ₅₀ VALUE
DPPH	Pet Ether	136.01±17
	Chloroform	155.19±35
	Ethyl Acetate	173.55±06
	Aqueous	52.75±29
Standard	Gallic acid	10.8±0.02

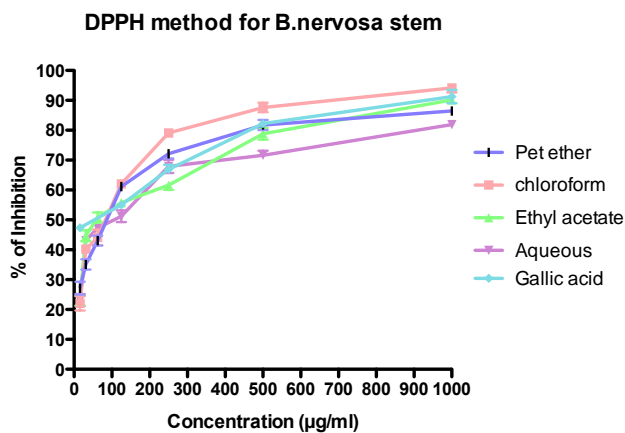


Figure: B. nervosa Stem

METHOD	FRACTIONS	IC ₅₀ VAULE
DPPH	Pet Ether	91.68±014
	Chloroform	98.38±217
	Ethyl Acetate	69.19±62
	Aqueous	120.58±40
Standard	Gallic acid	10.8±0.02

ABTS Method

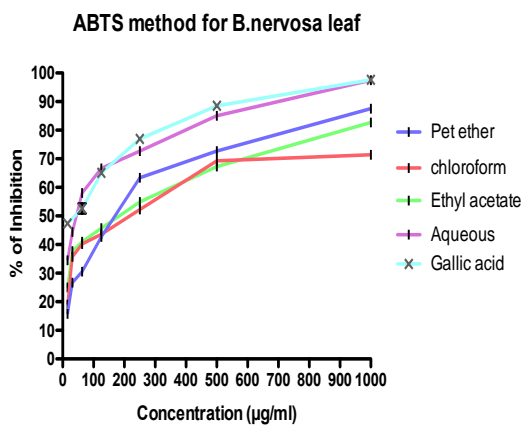


Figure: B. nervosa Leaf

METHOD	FRACTIONS	IC ₅₀ VAUL
ABTS	Pet Ether	178.79±59
	Chloroform	195.24±02
	Ethyl Acetate	196.80±011
	Aqueous	45.14±18
Standard	Gallic acid	10.8±0.02

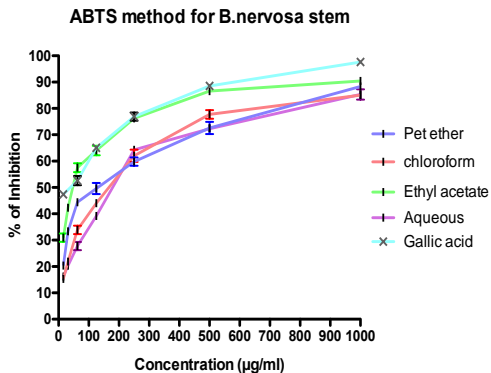


Figure: *B. nervosa* Stem

METHOD	FRACTIONS	IC50 VAULE
	Pet Ether	156.29±36
	Chloroform	195.97±20
ABTS	Ethyl Acetate	59.91±74
	Aqueous	209.87±09
Standard	Gallic acid	10.8±0.02

Nitrous Oxide (NO₂)

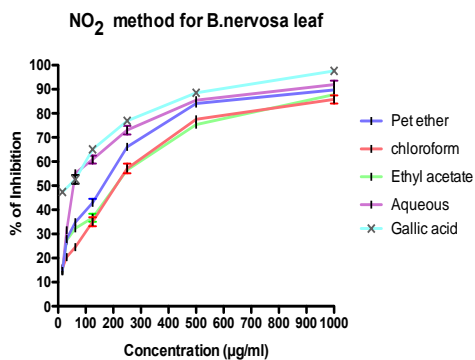


Figure: *B. nervosa* Leaf

METHOD	FRACTIONS	IC ₅₀ VAULE
NO ₂	Pet Ether	176.31±55
	Chloroform	211.46±17
	Ethyl Acetate	221.08±39
	Aqueous	64.32±74
Standard	Gallic acid	10.8±0.02

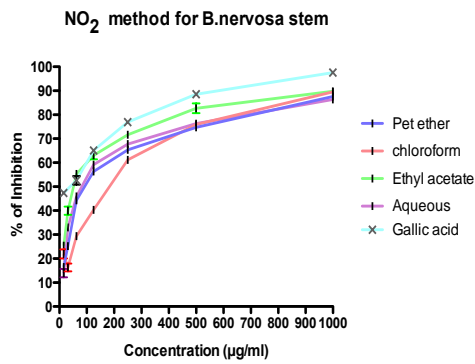


Figure: *B. nervosa* Stem

METHOD	FRACTIONS	IC ₅₀ VAULE
NO ₂	Pet Ether	64.57±29
	Chloroform	206.32±18
	Ethyl Acetate	54.30±31
	Aqueous	96.37±40
Standard	Gallic acid	10.8±0.02

Hydrogen Peroxide (H₂O₂)

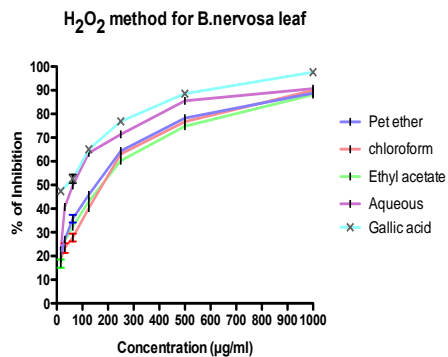


Figure: *B. nervosa* Leaf

METHOD	FRACTIONS	IC ₅₀ VAULE
H ₂ O ₂	Pet Ether	175.52±36
	Chloroform	197.59±27
	Ethyl Acetate	201.67±18
	Aqueous	70.80±93
Standard	Gallic acid	10.8±0.02

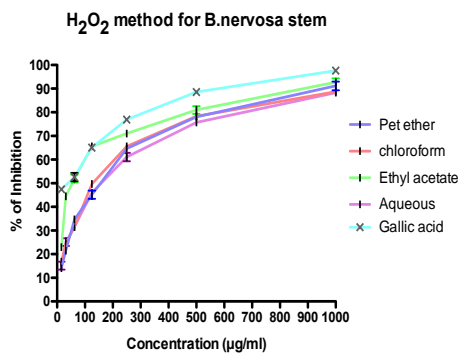


Figure: *B. nervosa* Stem

METHOD	FRACTIONS	IC50 VAULE
H2O2	Pet Ether	168.61±04
	Chloroform	149.77±54
	Ethyl Acetate	56.06±49
	Aqueous	171.67±81
Standard	Gallic acid	10.8±0.02

Conclusion

The preliminary photochemical screening results indicate that the presence of alkaloids, phenols, saponins, steroids and triterpenes were present in *B. nervosa* and carbohydrates flavonoids and lignins were present in lesser amount. The results of the experiments clearly suggested that *B. nervosa* leaf and stem extracts has potential anti- bacterial and anti-fungal agents against *E. coli* and *A. niger*. *B. nervosa* leaf and stem possessed potent antimicrobial activity against *E. coli* and *A. niger*. This scientific exploration will help to identify effective antimicrobial agents from medicinal plants, which may be clinically investigated for the treatment of infectious diseases. The *B. nervosa* leaf and stem fractions where subjected for antioxidant studies using DPPH, ABTS, Nitrous oxide and H₂O₂ and in all the anti-oxidant assays performed *B. nervosa* leaf showed good IC₅₀ value in Aqueous fraction and *B. nervosa* stem showed good IC₅₀ value in Ethyl acetate fraction. This scientific exploration will help to identify effective antimicrobial agents from *B. nervosa*, which may be clinically investigated for the treatment of infectious diseases.

Acknowledgement

The authors are very much thankful to Dr. S.P. Dhanabal and Dr. B. Duraiswamy for their support and to carry out the work. The authors are also thankful to the Department of Pharmacognosy, J.S.S college of Pharmacy, Nilgiris, India for financial support.

References

1. Afzal Azam, MD., et al. *Ancient Sci. Life*, **1998**. 18(2):112-119.
2. Anonymous, *The Wealth of India: A dictionary of Indian raw materials and Industrial products.* New Delhi, India: 6th Vol. Publication and Information Directorate (CSIR), **1962**.
3. Anonymous, *Ethnobiology in India: A status Report.* All India Co-ordinated Research Project in Ethnobiology. Ministry of Environment and Forests, New Delhi, Government of India, **2000**.
4. Easu, K., *Plant Anatomy.* John Wiley and sons, New York, **1965**.
5. Jackson, BP, and Snowdon, DW., *Powdered vegetable drugs.* Thames publishes, London, *Plant Micro technique.* Mc Graw hill book Co, New York, 1968.

6. Metcalfe, CR., and Chalk, L., Anatomy of the Dicotyledons, Clarendon Press, **1979**. 1, Oxford.
7. O'Brien, TP., et al *Protoplasma*, **1964**. 59: 364-373.
8. Rai, B., Khawas, DB., and Chettri, R., *J. Econ. Tax. Bot. Addl. Ser*, **1992**. 10: 193-198.
9. Rajan, S., Sethuraman, M., Folk Medicine of the Nilgiri Hills in Southern India. Survey of Medicinal Plants & Collection Unit and Hill area development programme, Udhagamandalam, **2008**. 1: 115-127.
10. Trease, GE., and Evans, WC., Pharmacognosy. Bailliere Tindal, London. Wallis TE. 1985. Text Book of Pharmacognosy. CBS Publishers and Distributors. Shahdara, Delhi, **1978**.
11. Wiegand, I., Hilpert, K., Hancock, REW., *Nature Protocols*. **2008**. 3(2): 163-175.
12. Trease, GE., and Evans, MC., Text book of pharmacognosy, (Balliere, London), Phytochemical methods: A guide to modern techniques of plant analysis, Chapman and Hall, London, **1983**.
13. National Committee for Clinical Laboratory Standards. Methods for Dilution Anti microbial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Seventh Edition, CLSI document M7-A7 (ISBN 1-56238-587-9), CLSI, Wayne, Pennsylvania 19087-1898 USA. **2006**.
14. Devi, KP., et al.. Pandian, *J Ethnopharmacol*, **2010**. 130(1): 107-15.
15. Zampini, IC., Vattuone, MA., and Isla, MI., *J Ethnopharmacol*, **2005**. 102(3): 450-6.
16. Burt, S., *Int J Food Microbiol*. **2004**. 94(3): 223-53.